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MORPHOMETRY OF KIDNEY BIOPSY: INFLUENCE OF SECTION THICKNESS VARIATION

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ABSTRACT

We studied the intraobserver variation in estimates of morphometric glomerular parameters when section thickness varied. Paraffin sections 1, 2, 3, 4, and 5 μm thick were cut in series of a kidney biopsy with an automatic electronically steered microtome. The sections were stained with Masson trichrome stain and glomeruli common to all five sections selected for analysis. One observer studied the sections five times to estimate the variation limits within one biopsy. As expected, the number of glomerular nuclei increased with increasing thickness of sections. In reference to the area of glomeruli the range of variation between the five sections was 41 per cent of the mean. Variation of individual estimates clearly increased with increasing thickness of section. The mean of the estimates of the volume fraction of the mesangium slightly increased with increasing thickness. In general, variation was least in thickness range of 1-3 μm. The variation in thicker sections seemed to be due to superimposition of structures which led to difficulties in interpretation. The surface density of glomerular basement membrane showed the smallest variation in sections 3 - 4 μm thick. This shows that certain measurements will not give the most reliable results in the thinnest sections.

INTRODUCTION

There is considerable potential for section thickness variation in diagnostic histopathology. In a histopathology laboratory sections are cut with different microtomes and by

different technicians, and an accepted tradition seems to define the thickness which is considered appropriate for each special purpose. No doubt the practices vary between different laboratories as do the methods of fixation, dehydration and embedding. Against this background one would like to know how variation in section thickness affects the variation ranges of morphometric results.

MATERIAL AND METHODS

Paraffin sections 1, 2, 3, 4, and 5 µm thick were cut in series of a kidney biopsy with an LKB automatic electronically steered microtome. Glass knives were used. We used adjacent sections so that the microscopic images of the sections contained the same tissue structures. The biopsies had been fixed in Bouin's fluid and embedded in paraffin (Paraplast). They were stained with Masson trichrome stain. Details of the method used have been published earlier (Romppanen and Collan 1981, Collan et al. 1982). Each section of the biopsy was studied five times by one observer and 30 different morphometric parameters were calculated for each section. Glomerular parameters were estimated of glomeruli which were common to all four sections.

The producer of the microtome gave an accuracy of +/- 10% for the thickness setting in metacrylate sections. For paraffin sections we estimated that variation was less than +/- 50% of the thickness setting. It is important to realise that there is no doubt about the fact that the variation range of section thickness within a laboratory and also between laboratories is smaller than from 1 to 5 µm.

RESULTS

Table 1 shows the results on 4 diagnostically relevant parameters. These parameters also present numerical density, volume density, surface density and length density measurements. The results are what could be expexted for the number of nuclei per glomerular area. Their number increases with section thickness. However, also the variation increases considerably after 3 µm sections. There is no dramatic increase in estimates of glomerular mesangium with increasing section thickness. Surface density of glomerular basement membrane and length density of glomerular capillaries decreased markedly in sections thicker than 3 µm. Table 2 shows how the results can be used to estimate the limits of possible performance in morphometry in cases in which there is variation in section thickness. It can be seen from the table that when a tolerance of 4 µm is accepted in section thickness a maximum variation of about 41% is to be expected in an estimate of glomeru-

Table 1.

Values of selected morphometric parameters; coefficient of variation (in percent) in parentheses.

	Section thickness (in μms)				
	1	2	3	4	5
No of nuclei per glomerular profile area, in mm^2	6337 (1.9)	6574 (1.9)	7724 (3.4)	8518 (5.1)	8936 (8.0)
Volume fraction of glomerular mesangium	11.1 (2.3)	11.7 (2.1)	13.7 (3.1)	13.5 (6.3)	12.7 (8.6)
Surface density of glomerular basement membrane	0.576 (4.9)	0.601 (6.8)	0.533 (2.5)	0.411 (1.9)	0.272 (11.0)
Length density of glomerular capillaries	28.6 (6.0)	31.3 (5.9)	30.3 (9.4)	23.8 (9.2)	18.0 (8.8)

lar nuclei. This means that the result 0.5 - 1.5 times the real value could occur under normal conditions. If the tolerance is less, the total range of results will be smaller, e.g. with tolerance of 1 μm the range of results is within values 0.8 - 1.2 times the real value. The maximum range of variation of mesangial volume fraction does not seem to change with increasing thickness of sections. On the other hand surface density of glomerular basement membrane will show considerable variation at higher tolerance levels.

Table 2.

Maximum range of variation (as % of the mean of all measurements) of three glomerular parameters measured with morphometry by a single observer at maximal tolerated variation (tolerance) limits in section thickness.

	Tolerance (in μms)			
	1	2	3	4
No of nuclei per glomerular profile area	17.5	22.8	37.3	41.0
Volume fraction of glomerular mesangium	17	23	23	23
Surface density of glomerular basement membrane	29	43	50	65

DISCUSSION

The morphometric measurement of the mesangial volume fraction was not much affected by the variation in section thickness. On the other hand the nuclear density was very much influenced. This suggests that small nuclear density differences are meaningless if section thickness is not reliably standardized. Several other parameters (surface density of the basement membrane and the length density of the glomerular capillaries) seem to change dramatically at section thickness higher than 3 um. This is probably due to the fact that thicker sections allow superimposition of structures and make distinction of perpendicular cross sections difficult or impossible.

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